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Original Article

Repurposing Pyrrolopyrimidine Schiff Base Transition Metal(II) Complexes for Anticancer Activity: Computational Insights into EGFR Inhibition

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ABSTRACT

Pyrrolopyrimidine scaffolds are well-recognized in the development of EGFR inhibitors and remain a privileged framework for anticancer drug discovery. Transition metal (II) complexes with tridentate Schiff bases derived from pyrrolopyrimidines have previously been explored for their antimalarial and antioxidant properties. However, their potential as anticancer agents, particularly EGFR inhibitors, has not been investigated. Based on this rationale, it is hypothesized that such complexes may also exhibit inhibitory activity against EGFR, and applied a computational repurposing strategy to assess their potential. Among the screened complexes, Compound 3 displayed favorable docking within the ATP-binding cleft of EGFR, establishing interactions with hinge and catalytic residues such as Phe723 and Cys797. To validate its stability under physiological conditions, a 200 ns molecular dynamics simulation was conducted, followed by analyses of RMSD, RMSF, ligand–protein contacts, PCA, FEL, and MM/GBSA calculations. The EGFR–Compound 3 complex showed a slight reduction in flexibility within certain functional regions compared with the apo form, suggesting a tendency toward local stabilization. This study provides the first evidence that pyrrolopyrimidine-based transition metal (II) complexes may act as potential EGFR inhibitors, highlighting their potential based on preliminary computational evidence and supporting further experimental validation.

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Introduction

Cancer is a formidable global health challenge. In 2022, nearly 20 million new cancer cases were reported globally, with approximately 9.7 million deaths [1,2]. This means that about one in five people develops cancer in their lifetime, while one in nine men and one in 12 women die from it [1]. Projections look even more daunting by 2050; new cancer cases are expected to soar to over 35 million annually, with deaths potentially doubling driven by population growth and aging [3,4]. The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that regulates cell proliferation, differentiation, and survival. Dysregulation via overexpression or activating mutations is significantly linked to carcinogenesis in various cancer types, including non-small cell lung cancer, breast cancer, colorectal cancer, and glioblastoma. Therefore, EGFR is a well-validated and valuable anticancer target [5,6]. Although EGFR-targeted therapies such as Gefitinib, Erlotinib, and Osimertinib have improved patient outcomes, their effectiveness is frequently hampered by concerns such as acquired resistance (*e.g.,* T790M and C797S mutations), toxicity, and limited efficacy in specific patient populations. To address these issues, novel scaffolds must be developed that may overcome resistance while remaining potent [7,8]. The pyrrolopyrimidine scaffold has emerged as a promising template in this regard, and its fused heterocyclic structure promotes strong interactions in the EGFR ATP-binding region. Various derivatives have demonstrated significant inhibition, including against mutant versions [9]. Schiff base transition metal complexes are known to have diverse biological activities, including anticancer, antimicrobial, and antioxidant effects. Their structural versatility allows fine-tuning of metal–ligand interactions, which can enhance target specificity and binding affinity, making them attractive candidates for targeted therapy. In addition, metal coordination can modulate potential side effects, providing a balance between efficacy and toxicity. Despite these advantages, few studies have explored the combination of this scaffold with transition-metal complexes, which may offer benefits such as increased stability, novel redox properties, and unique binding geometries that could improve target engagement. This study aimed to fill this gap by exploring the computational potential of pyrrolopyrimidine Schiff base transition metal (II) complexes as novel EGFR inhibitors. Through molecular docking and molecular dynamics simulations, the binding affinity, specificity, and conformational stability were probed within the EGFR active site, laying the groundwork for future experimental validation and anticancer development.

Rationale

Bagul *et al.* investigated the antimalarial and antioxidant properties of transition metal (II) complexes derived from pyrrolopyrimidine-based Schiff bases [10]. While their research concentrated on parasite and oxidative stress-related actions, it is worth noting that pyrrolopyrimidine was previously recognized as a preferred scaffold for anticancer activity, specifically as an EGFR inhibitor [11-16]. Inspired by these findings, it is posited that the pyrrolopyrimidine Schiff base transition metal (II) complexes disclosed in Bagul's paper would potentially be useful EGFR inhibitors. It is notable that no previous study has assessed their efficacy against EGFR in the context of cancer therapy. In this study, these complexes are considered for anticancer activity using a computational method that includes molecular docking and molecular dynamics simulations. This approach evaluates their binding affinity, stability, and potential as EGFR-targeted anticancer drugs, opening new avenues for the use of pyrrolopyrimidine-based metal complexes in oncology.

Materials and Methods

Molecular docking studies

The EGFR protein structure (PDB ID: 8SC7) was created according to a standard protein

preparation workflow [17]. Water molecules that extended beyond the defined cutoff were removed and appropriate bond orders and protonation states at physiological pH (7.4) were assigned. Missing loops and side chains were modeled and the hydrogen-bonding network was improved. To eliminate steric clashes, the structure underwent restrained minimization. ChemDraw was used to generate the ligands. Before importing the molecule into Schrödinger Maestro, the coordination bonds of the metal complex were represented as standard covalent bonds [18-21]. The bonds between the central metal atom and the surrounding ligands were reassigned as coordination interactions in the workspace by selecting the "create zero-order bonds to metal" option from the build menu [22,23]. The “zero-order bond” representation provides an approximate description of the metal–ligand coordination environment. This approach was adopted to preserve the experimentally derived coordination geometry, while allowing compatibility with the docking protocol. Ligand structures were then energy-minimized using the minimized function in the edit menu to complete their preparation. For docking studies, the grid was centered on the EGFR active site, with the co-crystallized ligand as a reference. Molecular docking was performed using Glide in the standard precision (SP) mode, and the resulting poses were evaluated based on docking scores and refined using post-docking minimization [24].

Molecular dynamics simulations

In molecular dynamics (MD) simulations, the protein-ligand complex was solved in an explicit water box with a 10 Å buffer. The system was neutralized and supplemented with 0.15 M NaCl to simulate physiological conditions. The OPLS3e force field was applied to both the protein and ligand using built-in parameterization tools. Following an equilibration phase in the NVT and NPT ensembles with positional restraints gradually released, a 200 ns production simulation was run at 300 K and 1 atm. The temperature and pressure were controlled by a Nose-Hoover thermostat and an appropriate barostat. A 2-fs time step with hydrogen bond constraints was used, and trajectory frames were saved at 10-50 ps intervals for later analysis [25-27]. The 200 ns simulation provided meaningful insights into the short- to mid-term stability and key interaction patterns of the EGFR–Compound 3 complex; however, longer simulations are required to capture slower conformational transitions and achieve thermodynamic convergence.

Conformational dynamics and energy landscapes

Principal component analysis (PCA) was used to investigate the collective motion contributing to protein conformational dynamics. Additionally, free energy landscapes (FELs) were generated to examine the stability and folding behavior of the complexes. Conformational sampling applied for FEL construction allowed a deeper understanding of the dynamic stability of protein-ligand systems throughout the simulation. Both the PCA and FEL calculations were performed using the trj_essential_dynamics script in Schrödinger [28,29].

Binding free energy calculations

The binding free energy of the protein-ligand complexes was calculated using the molecular mechanics/generalized Born surface area (MM/GBSA) method. Trajectories from molecular dynamics simulations (0-200 ns) were recovered and processed using the Schrödinger's Prime module. The *thermal mmgbsa.py* script was used to calculate the relative binding free energies, which included molecular mechanics energies (electrostatic and van der Waals), solvation contributions, and surface area terms. This technique provides a more realistic measurement of binding affinity than docking scores alone because it accounts for both enthalpic and solvation effects collected during the simulation [30,31].

Results and Discussion

Transition metal (II) complexes with tridentate Schiff bases derived from pyrrolopyrimidine have previously been explored by Bagul *et al.* for their antimalarial and antioxidant properties [10]. However, their potential as anticancer agents, particularly epidermal growth factor receptor (EGFR) inhibitors, has not yet been investigated. Since pyrrolopyrimidine scaffolds are well recognized in the design of anticancer molecules, it is hypothesized that these complexes may also exhibit inhibitory activity against EGFR. To explore this possibility, a computational repurposing approach and performed molecular docking of compounds 1-5 with EGFR were employed.

Docking result analysis

Molecular docking of pyrrolopyrimidine-derived transition metal complexes (**Compounds 2-5**) with the EGFR active site was performed, and the results are summarized in **Table 1**.

The docking scores ranged from -6.819 to -8.145 kcal/mol, while MM/GBSA ΔGBind values ranged from -19.74 to -91.35 kcal/mol, demonstrating significant variations in binding affinities between these complexes. The free ligand, PPHmCB (Compound 1), had a docking score of -7.401 kcal/mol and a ΔGBind of -51.09 kcal/mol. $Cd(PPHmCB)_2$ (Compound 2) has a favorable ΔGBind (-52.23 kcal/mol) and interacts ionically with Asp800 (**Figure 1**).

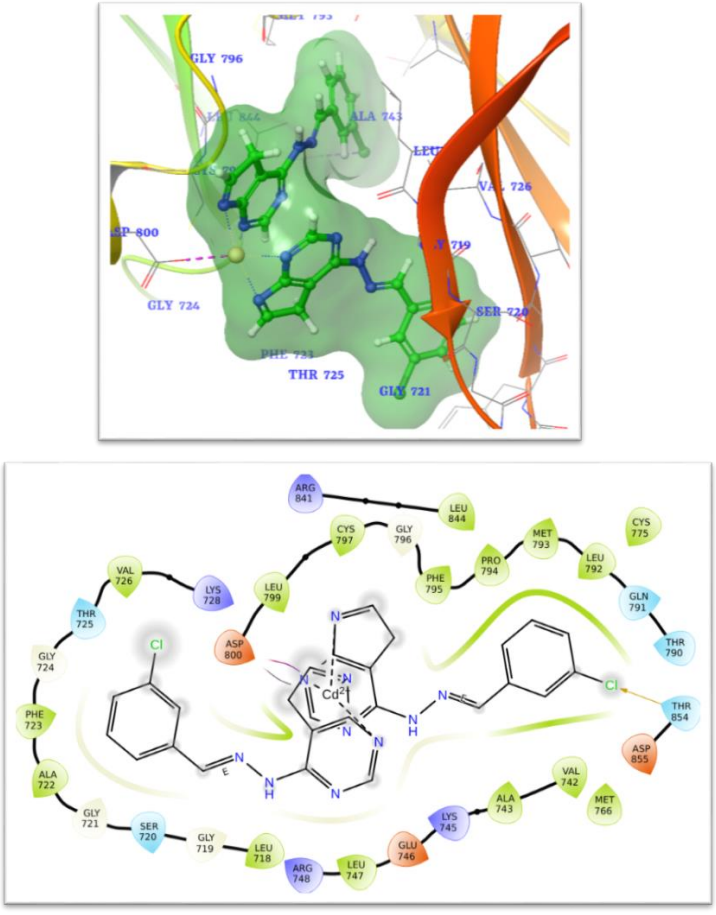
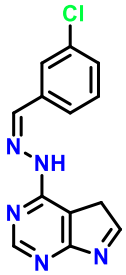
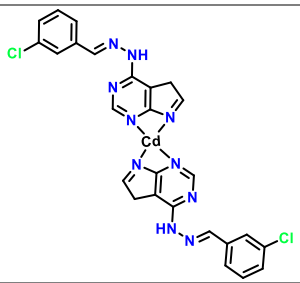
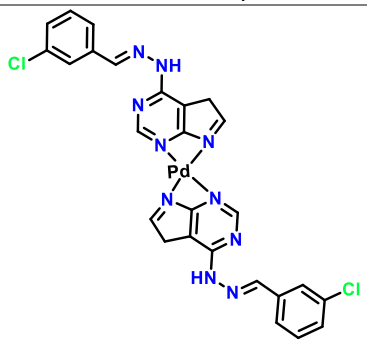
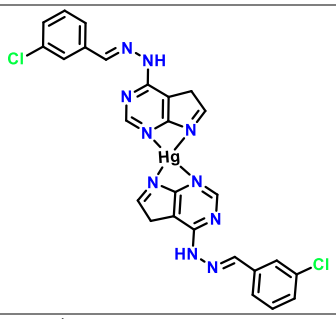
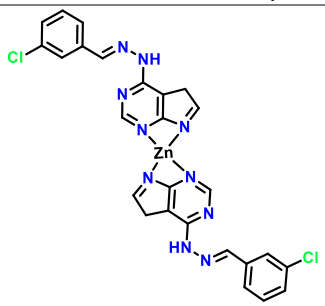


Figure 1. 2D and 3D binding interactions of $Cd(PPHmCB)_2$ (Compound 2) in the EGFR kinase domain (PDB ID: 8SC7)

Table 1. Docking scores, Glide emodel, MM/GBSA binding free energies (ΔGBind), and Prime energies of pyrrolopyrimidine-derived transition metal (II) complexes with EGFR (PDB ID: 8SC7)

Compound No	Structure	Compound name	Docking score	Glide emodel	MMGBSA ΔG Bind	Prime energy
1		PPHmCB	-7.401	-63.892	-51.09	-13950.9
2		$Cd(PPHmCB)_2$	-6.992	-94.874	-52.23	-13982.3
3		$Pd(PPHmCB)_2$	-8.145	-100.269	-71.85	-13990.6
4		$Hg(PPHmCB)_2$	-7.107	-91.331	-19.74	-14064.2
5		$Zn(PPHmCB)_2$	-6.819	-93.985	-91.35	-14019.9

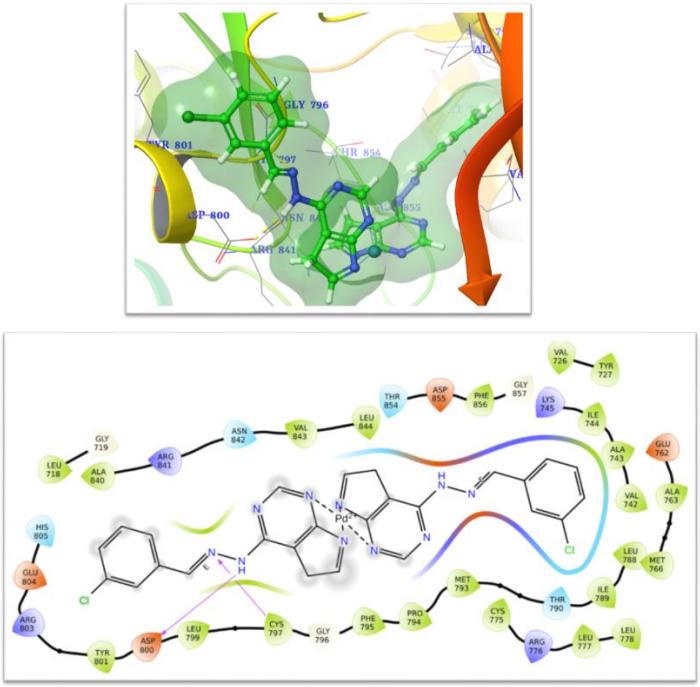


Figure 2. 2D and 3D binding interactions of $Pd(PPHmCB)_2$ (Compound 3) in the EGFR kinase domain (PDB ID: 8SC7)

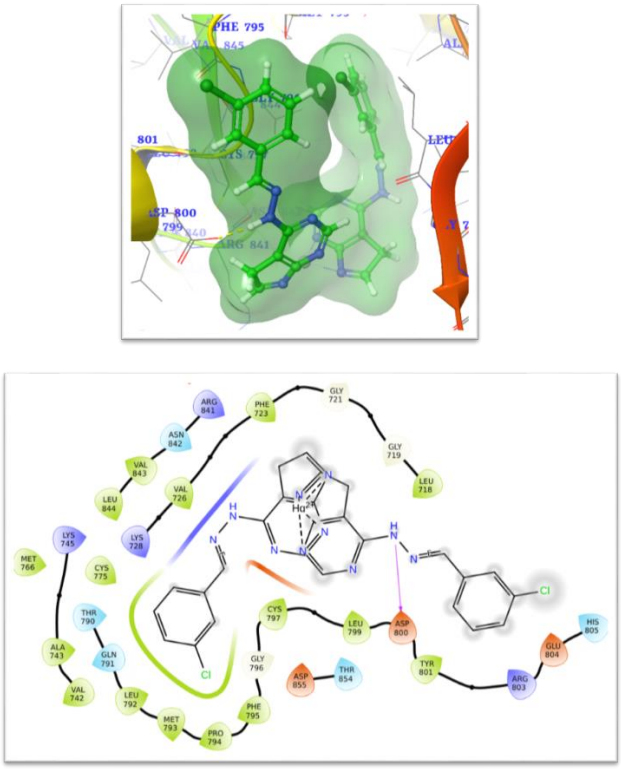


Figure 3. 2D and 3D binding interactions of $Hg(PPHmCB)_2$ (Compound 4) in the EGFR kinase domain (PDB ID: 8SC7)

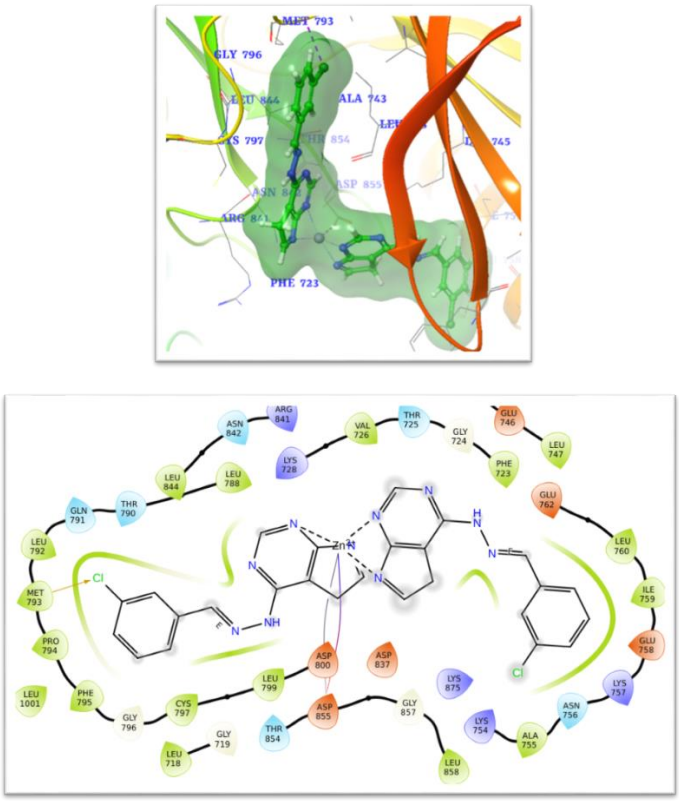


Figure 4. 2D and 3D binding interactions of $Zn(PPHmCB)_2$ (Compound 5) in the EGFR kinase domain (PDB ID: 8SC7)

The docking score (-6.992 kcal/mol) was slightly lower than that of $Pd(PPHmCB)_2$. However, contact with Asp800 suggests a possible inhibitory activity, as this residue is critical for stabilizing the active DFG-in conformation of EGFR. $Pd(PPHmCB)_2$ (Compound 3) had the best docking profile, with a score of -8.145 kcal/mol and a ΔGBind of -71.85 kcal/mol. This compound also had the lowest Glide emodel (-100.269 kcal/mol), indicating a solid accommodation inside the binding pocket. $Pd(PPHmCB)_2$ formed two critical interactions: (i) the secondary amine group established hydrogen bonding with Asp800 of the conserved DFG motif, and (ii) the Schiff base nitrogen interacted with Cys797, a covalent gatekeeper residue commonly targeted by clinically approved irreversible EGFR inhibitors (*e.g.,* osimertinib). This dual engagement increases the binding selectivity and may lead to effective kinase inhibition (**Figure 2**). $Hg(PPHmCB)_2$ (Compound 4) has moderate binding (-7.107 kcal/mol; ΔGBind = -19.74 kcal/mol) and forms a hydrogen bond with Asp800 via its secondary amine group (**Figure 3**, **Table 2**). The lower binding free energy suggests that the complex is less stable within the pocket than $Pd(PPHmCB)_2$. $Zn(PPHmCB)_2$ (Compound 5) had a lower docking score (-6.819 kcal/mol) but the best MM/GBSA binding free energy (-91.35 kcal/mol). These chemical forms an ionic contact with Asp855, a residue in the activation loop (A-loop) of EGFR. Asp855 is important for stabilizing conformational changes in the kinase. Thus, this contact may contribute to long-range conformational regulation despite the lower docking score (**Figure 4**). Additionally, a halogen bond was observed between the chlorophenyl group of the ligand and hinge region residue Met793, which is a critical anchoring point for many EGFR inhibitors. The docking results showed that Pd $(PPHmCB)_2$ (Compound 3) was the most promising candidate.

Table 2. Comparative Binding interaction analysis of pyrrolopyrimidine-based transition metal (II) complexes with EGFR (PDB ID: 8SC7)

Compound	Key Residue interactions	Significance
Compound 1 (PPHmCB, free ligand)	-	Reference ligand for comparison
Compound 2 ($Cd(PPHmCB)_2$)	Ionic with Asp800	Slightly lower docking score than Pd complex; interaction with DFG Asp800 suggests inhibitory potential
Compound 3 ($Pd(PPHmCB)_2$)	H-bond with Asp800; Schiff base N with Cys797	Best docking score and Glide emodel; dual interactions with key residues enhance selectivity and potential kinase inhibition
Compound 4 ($Hg(PPHmCB)_2$)	H-bond with Asp800	Moderate binding; lower ΔGbind suggests less stable pocket accommodation
Compound 5 ($Zn(PPHmCB)_2$)	Ionic with Asp855	The best ΔGbind; interaction with activation loop residue may affect long-range conformational regulation despite lower docking score

Its combined interactions with Asp800 of the DFG motif and Cys797, a crucial residue for covalent inhibition, substantially promoted its efficacy as an EGFR inhibitor. Previous research has confirmed Asp800's critical role in catalysis and activation loop dynamics, whereas Cys797 is the key nucleophilic site targeted by next-generation irreversible EGFR inhibitors. The ability of $Pd(PPHmCB)_2$ to engage both residues suggests that pyrrolopyrimidine-based metal complexes could be suitable scaffolds for developing novel EGFR-targeted anticancer drugs.

Analysis of MD trajectories

A 200 ns molecular dynamics (MD) simulation was performed to investigate the binding stability of the complex under physiological conditions. Various statistical parameters were used to evaluate the stability and dynamic behavior of the simulation, including the root mean square deviation (RMSD) to assess the overall structural stability, the root mean square fluctuation (RMSF) to monitor the flexibility of individual residues, and the ligand-protein contact profile to examine the persistence of key binding interactions. The RMSD profile revealed the overall structural stability of both the Apo form of EGFR and its complex with Compound 3 during the 200 ns MD simulation (**Figure 5**). The RMSD values of the EGFR Apo protein ranged from 1.24 Å to 3.00 Å, with an average of 2.52 Å. Similarly, the Compound 3-EGFR complex had a minimum RMSD of 1.21 Å, maximum of 3.02 Å, and average of 2.51 Å. Both systems showed an initial increase in RMSD within the first 10 ns due to equilibration and conformational changes. After an initial fluctuation, the trajectories of both Apo and ligand-bound systems stabilized, with values mostly within the 2.3-2.8 Å range.

The comparable average RMSD values of the Apo protein and the complex showed that ligand interactions did not cause major structural variations or instability in the EGFR backbone. In fact, the RMSD profile of the complex showed slightly smoother oscillations after equilibration than the Apo form, implying that Compound 3 binding may have helped stabilize the protein structure. Neither trajectory surpassed 3.1 Å, indicating no significant structural changes or unfolding. These data show that EGFR is structurally stable both in its free form and in complex with Compound 3, with the ligand retaining a stable binding mode throughout the simulation (**Figure 6**).

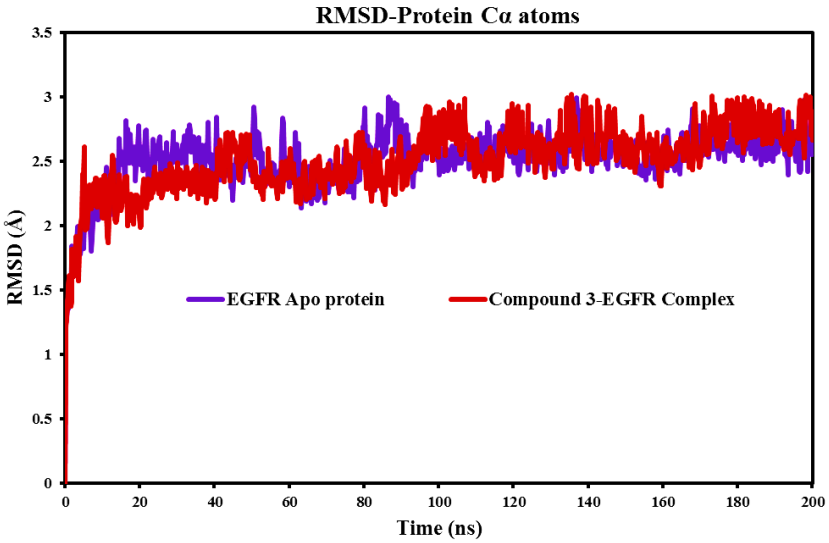


Figure 5. Time dependent RMSD of EGFR Apo Protein and in complex with Compound 3

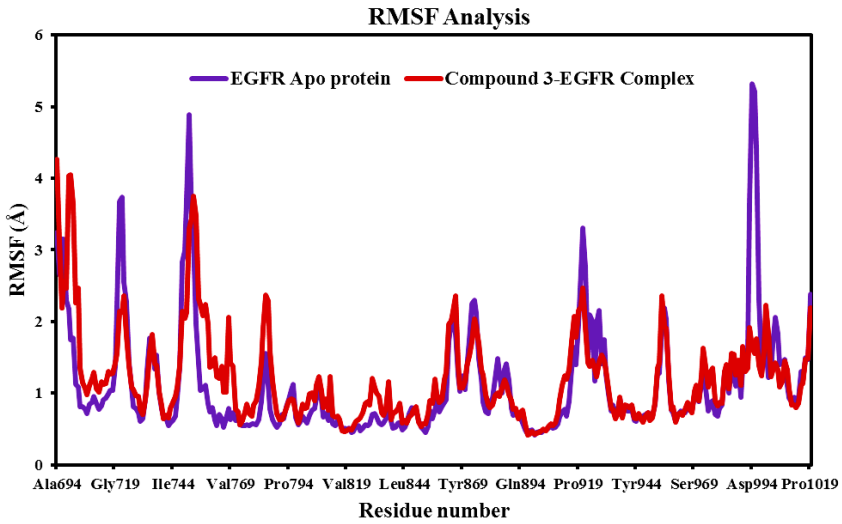


Figure 6. Individual amino acids RMSF of EGFR Apo Protein and in complex with Compound 3

The root-mean-square fluctuation is an important metric in molecular dynamics that quantifies how far each atom or residue deviates from its typical position over the course of a trajectory. It provides a clear indication of flexibility: low RMSF values suggest hard, stable sections, such as protein cores or well-ordered secondary structures, while high RMSF values indicate flexible parts, such as loops, termini, or disordered segments. The RMSF provided in **Figure 6** includes per-residue RMSF values (in Å) for both systems, enabling a direct comparison of residue-level flexibility between the Apo form and the ligand-bound complex. The Apo protein had RMSF values ranging from 0.42 Å to 5.32 Å, with an average of 1.12 Å, while the Compound 3-EGFR complex had values between 0.41 Å and 4.27 Å, with an average of 1.20 Å. Although the mean fluctuation is slightly larger in the complex, the overall profile indicates that ligand binding lowers excessive mobility in specific locations compared to the Apo form. Higher variations were detected in the Apo protein at residues

Asp994, Ser995, Thr751, Asn996, Ala722, Ala750, Gly721, Thr993, Ser752, Ser921, Ala694, Gly696, and Glu697, which were largely located in the flexible loop and terminal segments. These areas are naturally dynamic, and their mobility corresponds to the structural flexibility of the loops and termini in kinase proteins. Importantly, Compound 3 was linked to reduced fluctuations near the ligand-binding region. Compound 3 maintained stable interactions with key residues, including Leu718, Ser720, Phe723, Val726, Ala743, Ile744, Lys745, Glu762, Met766, Leu777, Leu788, Pro794, Phe795, Cys797, Leu799, Asp800, Tyr801, Arg803, Arg841, Asn842, Leu844, Thr854, Asp855, Phe997, and Ala1000, throughout the simulation. The RMSF values for these residues ranged from 0.69 Å to 2.35 Å, indicating moderate flexibility and productive binding interactions rather than disruptive fluctuations. Notably, several of these residues (Lys745, Cys797, Asp855, and Phe997) are known to play critical roles in EGFR kinase activity and inhibitor recognition, implying that Compound 3 maintains the active site environment during simulation. Taken together, the RMSF results suggest that Compound 3 binding limits excessive flexibility in crucial functional areas of EGFR, improving the structural stability of the protein-ligand complex. Ligand-protein contact analysis was performed to determine the nature and persistence of contacts that stabilized the Compound 3-EGFR complex during the 200 ns MD simulation. The contact histogram (**Figure 1**) and 2D ligand interaction patterns showed that the ligand generated many stable connections within the ATP-binding pocket of EGFR. Phe723 was identified as a significant residue, accounting for 61% of hydrophobic interactions with the pyrimidine ring of the ligand. This highlights the relevance of π–π stacking and van der Waals forces in stabilizing Compound 3's aromatic moiety of 3. Similarly, Cys797, a residue located in the hinge region and widely recognized as a critical anchoring point for many EGFR inhibitors, engages in hydrophobic and π-electron interactions with the pyrimidine ring nitrogen for 49% of the total simulation time, emphasizing its importance in maintaining a stable binding pose (**Figure 7**).

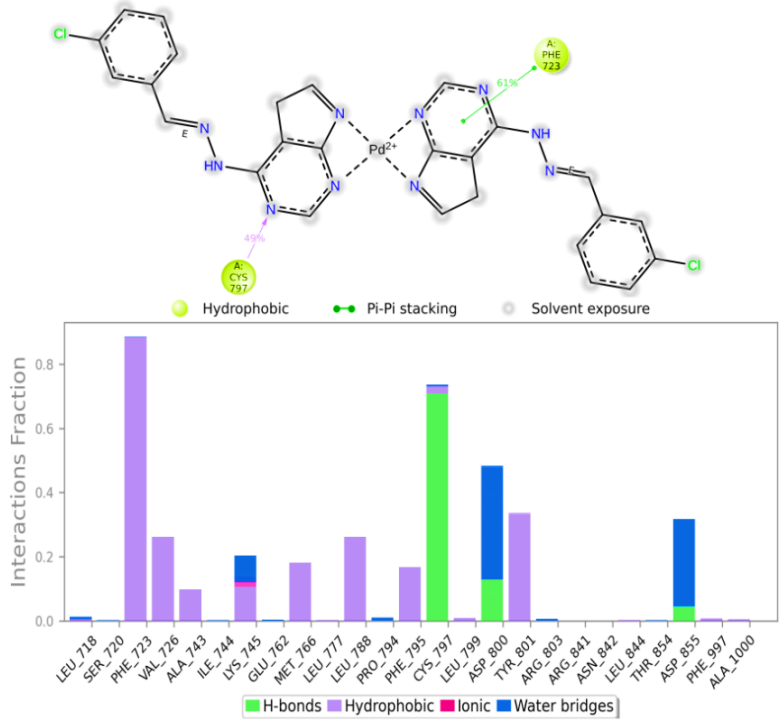


Figure 7. Ligand Contacts Histogram of Compound 3 in complex with EGFR

The histogram results confirmed that hydrophobic residues, such as Val726, Ala743, Met766, Leu788, Phe795, and Tyr801, significantly contributed to ligand stability via constant nonpolar interactions. These hydrophobic interactions are critical for kinase inhibition because they decrease the solvent exposure of the ligand and provide a tight fit within the binding pocket. In addition to hydrophobic stability, water-mediated hydrogen bonds were identified between residues Asp800 and Asp855, which are part of the conserved DFG motif and activation loop, respectively. Such water bridges frequently improve the flexibility and endurance of hydrogen-bonding networks in dynamic settings, thereby increasing the ligand affinity. A small fraction of ionic interactions was also found with Lys745, a residue typically implicated in the electrostatic stabilization of kinase inhibitors. These findings suggest that Compound 3 maintains a stable binding state via a synergistic network of hydrophobic contacts, water-mediated hydrogen bonds, and an occasional ionic connection. The prevalence of hydrophobic contacts at important residues, such as Phe723 and Cys797, emphasizes the relevance of aromatic stacking and hinge-binding interactions, although auxiliary polar and ionic connections are anticipated to strengthen the complex and contribute to its potential inhibitory activity.

Conformational dynamics and thermodynamic features

Principal component analysis (PCA)

PCA was applied to capture the large-scale collective motions of EGFR in its Apo state and when bound to Compound 3 during the 200 ns molecular dynamics simulation. By projecting the trajectories onto the first two principal components (PC1 and PC2), the conformational space sampled by the systems can be viewed, providing information about structural flexibility and dynamic behavior. The scatter plot shows that the EGFR Apo protein (purple) has a greater conformational space than the Compound 3-EGFR complex (red). This larger distribution suggests greater conformational diversity in the absence of the ligand, which is consistent with the inherent flexibility of kinase domains, particularly in the loop and activation regions. In contrast, the Compound 3-EGFR complex has a more compact clustering of data points, implying that the ligand binding limits protein motion and stabilizes the protein in a tight conformational ensemble. This constraint in conformational sampling is frequently associated with successful ligand binding and the stability of the active or inhibited conformation of kinases. Interestingly, while there is considerable overlap between the Apo and complex distributions, the complex samples different parts of the conformational landscape than that of the Apo protein. This suggests that Compound 3 causes minor conformational changes in EGFR, possibly improving the shape of the binding pocket for stable interactions (**Figure 8**). The lower mobility observed in the complex was consistent with the RMSD and RMSF statistics, which also indicated crucial residue stability upon ligand binding. This supports the hypothesis that Compound 3 contributes to the conformational stabilization of EGFR, which is an important characteristic of its potential inhibitory activity.

Free energy landscape (FEL) analysis

During the 200 ns simulation, the Free Energy Landscape (FEL) was created to investigate the conformational stability and thermodynamic preferences of EGFR both in Apo and in combination with Compound 3. FEL maps the free energy surface as a function of RMSD and radius of gyration (Rg), emphasizing the conformational states observed in low-energy basins. The color gradient in the FEL graph extends from blue (lowest free energy and highly stable conformations) to green, yellow, and then red (higher free energy, less stable conformations). The blue patches represent the most favorable conformational states collected during the trajectory.

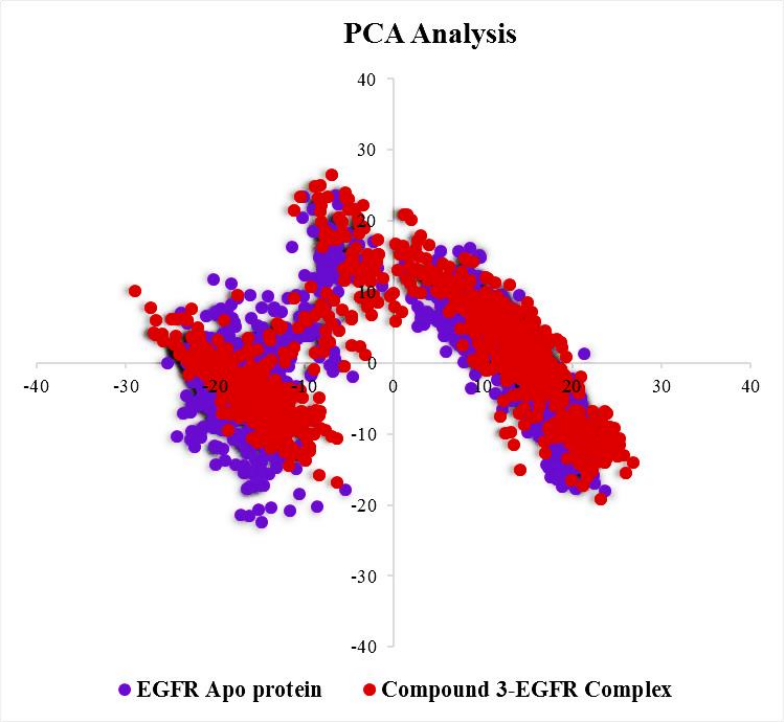


Figure 8. Projection of the motion for Cα atoms Apo EGFR protein and in complex Compound 3 data from obtained MD simulation trajectories

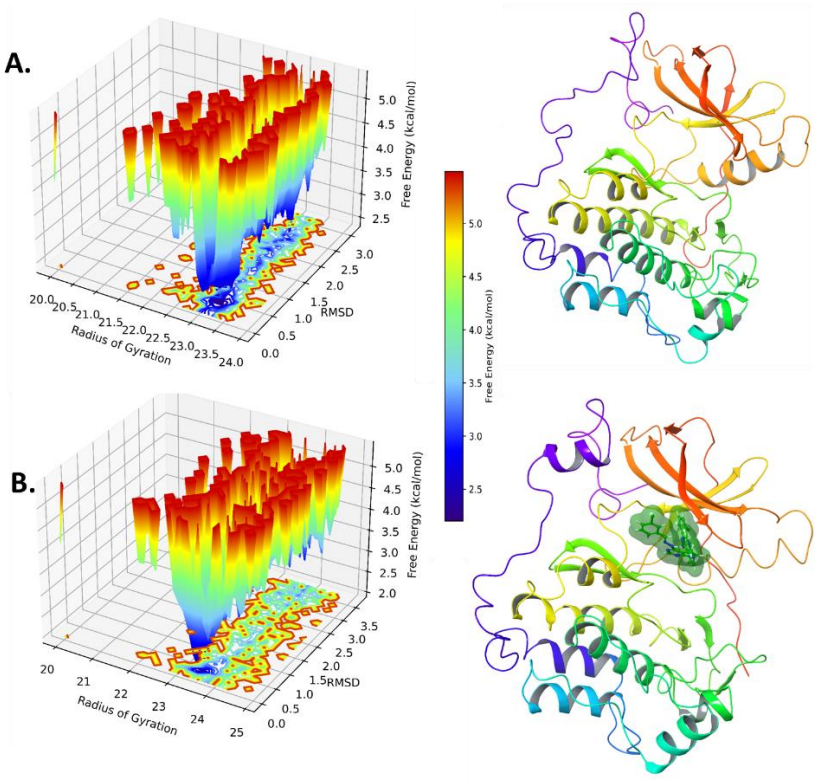


Figure 9. Free Energy Landscape (FEL) of EGFR Apo protein and Compound 3–EGFR complex

The study revealed a local minimum for the Compound 3-EGFR complex at frame 720, with an Rg of 23.245 Å and an RMSD of 1.57 Å. This suggests a relatively stable conformational state with tight folding and moderate structural variation from the initial conformation. The EGFR Apo protein showed a significant local minimum at frame 604, with an Rg of 22.512 Å and RMSD of 1.24 Å (**Figure 9**). The smaller RMSD of the Apo protein suggests structural stability; however, its energy distribution appears more scattered than that of the complex, which has compact and deeper minima. This indicates that Compound 3 reduces the conformational space, favoring fewer but more energetically stable states.

MM/GBSA-based binding free energy evaluation

The MM/GBSA approach was used to calculate the binding free energy of compound 3 with EGFR and to dissect the energetic contributions stabilizing the protein–ligand complex. The computed average binding free energy (ΔGbind) was -24.28 ± 15.35 kcal/mol, indicating that complex formation is thermodynamically favorable throughout the 200 ns simulation. Among the energy components, van der Waals interactions (ΔGbindvdw: -50.18 ± 9.72 kcal/mol) contributed most significantly to stabilization, highlighting the role of tight packing and hydrophobic contacts within the binding pocket. This observation aligns with the contact analysis, where hydrophobic residues such as Phe723, Val726, Ala743, Met766, Leu788, and Phe795 dominated the ligand binding. Lipophilic interactions (ΔGbindLip: -18.27 ± 5.55 kcal/mol) also contributed substantially, emphasizing the importance of hydrophobic stabilization. Electrostatic contributions (ΔGbindCoul: -241.34 ± 30.71 kcal/mol) were strongly favorable, reflecting polar interactions and ionic contacts with key residues, including Lys745, Asp800, and Asp855. The positive solvation free energy (ΔGbindsolGB: +284.18 ± 30.80 kcal/mol) partially countered these favorable interactions, which is consistent with the typical desolvation penalties in kinase–ligand complexes. Despite this, the net electrostatics remained favorable due to the strong hydrogen bonding and ionic interactions. Hydrogen-bonding contributions (ΔGbindHbond: -0.70 ± 0.54 kcal/mol) were modest but consistent with water-mediated hydrogen bonds observed in the contact analysis. Across the trajectory, ΔGbind ranged from -46.70 to ± 9.95 kcal/mol, indicating transiently weaker binding states, while the majority of conformations remained energetically favorable. Overall, these results show that compound 3 forms a persistent and energetically favorable binding state within the EGFR active site. The combination of van der Waals, lipophilic, and electrostatic interactions highlights the balanced interplay between hydrophobic packing and polar contacts as the main driving forces for binding. All MM/GBSA values were reported in kcal/mol with standard deviations to capture trajectory fluctuations, providing a comprehensive picture of the thermodynamic stability of the complex (**Table 3**).

Table 3. MM/GBSA-based binding free energy components for the Compound 3–EGFR complex

MM/GBSA parameter	ΔG_{Bind}	$\Delta G_{BindCoul}$	$\Delta G_{BindHbond}$	$\Delta G_{BindLip}$	$\Delta G_{bindsol\_GB}$	$\Delta G_{bindvdw}$
Maximum	-46.70	-331.13	-2.29	-26.47	205.84	-64.46
Minimum	9.95	-160.60	0.00	-7.23	371.62	-26.74
Average	-24.28	-241.34	-0.70	-18.27	284.18	-50.18
Std	15.35	30.71	0.54	5.55	30.80	9.72

Conclusion

The present study provides a comprehensive computational investigation of the unexplored potential of transition metal (II) complexes with tridentate Schiff bases derived from pyrrolopyrimidine as anticancer agents targeting EGFR. Although pyrrolopyrimidine scaffolds are widely recognized for the development of EGFR inhibitors, their transition metal–based derivatives have not yet been evaluated in this context. By adopting a computational repurposing strategy, Compound 3 was identified as a promising inhibitor candidate with strong binding affinity within the ATP-binding pocket of EGFR. Docking studies revealed key interactions between hinge and catalytic residues, including Phe723 and Cys797, which are critical for kinase inhibition. The stability of Compound 3 was further validated by 200 ns molecular dynamics simulations, which demonstrated consistent RMSD and reduced RMSF values compared to the Apo protein, suggesting a stable and less flexible binding conformation. Ligand–protein contact analysis confirmed persistent hydrophobic interactions and water-mediated hydrogen bonds with catalytically important residues. Principal component analysis (PCA) indicated that ligand binding confined EGFR dynamics to a more stable conformational space, while free energy landscape (FEL) mapping revealed distinct low-energy basins, highlighting favorable thermodynamic states. The MM/GBSA free energy calculations provided additional support for strong binding, with van der Waals and electrostatic contributions being the dominant stabilizing forces. Taken together, these results suggest that Compound 3 may act as a potential EGFR inhibitor. This study broadens the scope of pyrrolopyrimidine-based metal complexes and provides preliminary computational evidence to support their future evaluation in experimental anticancer studies.

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Conflict of Interest

Authors have declared that there is no conflict of interest exists.

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References

[1]. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., Jemal, A., Global cancer statistics 2022: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, **2024**, 74(3), 229-263.
[2]. Ori, M.O., Ekpan, F.M., Samuel, H.S., Egwuatu, O.P., Integration of artificial intelligence in nanomedicine. *Eurasian Journal of Science and Technology*, **2024**, 4(2), 88-104.
[3]. Li, L., Ye, R., Li, Y., Pan, H., Han, S., Lu, Y., Targeting TNFR2 for cancer immunotherapy: Recent advances and future directions. *Journal of Translational Medicine*, **2024**, 22(1), 812.
[4]. Siegel, R.L., Kratzer, T.B., Giaquinto, A.N., Sung, H., Jemal, A., Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians*, **2025**, 75(1), 10. *75*(1), 10-45.
[5]. Ahmad, I., Patel, H.M., From challenges to solutions: A review of fourth-generation egfr

tyrosine kinase inhibitors to overcome the c797s triple mutation in non-small cell lung cancer. *European Journal of Medicinal Chemistry*, **2024**, 117178.
[6]. Ahmad, I., Patel, H.M., Orthoallosteric egfr-tkis: A new paradigm in nsclc treatment strategy targeting the c797s mutation. *Drug Development Research*, **2025**, 86(1), e70036.
[7]. Ahmad, I., Patel, H.M., Repurposing non-nucleosidic reverse transcriptase inhibitors (nnrtis) to overcome egfr t790m-mediated acquired resistance in non-small cell lung cancer. *Journal of Cellular Biochemistry*, **2025**, 126(1), e30653.
[8]. Ahmad, I., Patel, H., Design, synthesis, and evaluation of novel 2, 4-disubstituted pyrimidine derivatives as double mutant epidermal growth factor receptor-l858r/t790m tyrosine kinase inhibitors. *Journal of Biochemical and Molecular Toxicology*, **2025**, 39(1), e70077.
[9]. Sanglikar, G., KumarTengli, A., A critical review on purine and pyrimidine heterocyclic derivatives and their designed molecules in cancer. *Results in Chemistry*, **2025**, 102210.
[10]. Bagul, A.D., Kumar, M., Alanazi, A.M., Tufail, A., Tufail, N., Gaikwad, D.D., Dubey, A., Experimental and computational evaluation of anti-malarial and antioxidant potential of transition metal (ii) complexes with tridentate schiff base derived from pyrrolopyrimidine. *BioMetals*, **2024**, 37(6), 1713-1737.
[11]. Madhurya, M.S., Thakur, V., Dastari, S., Shankaraiah, N., Pyrrolo [2, 3-d] pyrimidines as potential kinase inhibitors in cancer drug discovery: A critical review. *Bioorganic Chemistry*, **2024**, 153, 107867.
[12]. Zhang, Y., Gao, L., Design and discovery of novel pyrazole-pyrrolopyrimidine derivatives as anti-glioma agents via promoting apoptosis, inhibiting cell cycle and egfr-tk. *Chemical Biology & Drug Design*, **2023**, 102(5), 1248-1256.
[13]. Seyed Hosseini, S.V., Pain management strategies and general condition of patients after intestinal cancer surgeries. *Eurasian Journal of Science and Technology*, **2025**, 5(1), 1-16.
[14]. Xia, Z., Huang, R., Zhou, X., Chai, Y., Chen, H., Ma, L., Yu, Q., Li, Y., Li, W., He, Y., The synthesis and bioactivity of pyrrolo [2, 3-*d*] pyrimidine derivatives as tyrosine kinase inhibitors for nsclc cells with egfr mutations. *European Journal of Medicinal Chemistry*, **2021**, 224, 113711.
[15]. Yadav, T.T., Moin Shaikh, G., Kumar, M.S., Chintamaneni, M., Yc, M., A review on fused pyrimidine systems as egfr inhibitors and their structure–activity relationship. *Frontiers in Chemistry*, **2022**, 10, 861288.
[16]. Lategahn, J., Keul, M., Klövekorn, P., Tumbrink, H.L., Niggenaber, J., Müller, M.P., Hodson, L., Flaßhoff, M., Hardick, J., Grabe, T., Inhibition of osimertinib-resistant epidermal growth factor receptor egfr-t790m/c797s. *Chemical Science*, **2019**, 10(46), 10789-10801.
[17]. Whitehead, C.E., Ziemke, E.K., Frankowski-McGregor, C.L., Mumby, R.A., Chung, J., Li, J., Osher, N., Coker, O., Baladandayuthapani, V., Kopetz, S., A first-in-class selective inhibitor of egfr and pi3k offers a single-molecule approach to targeting adaptive resistance. *Nature cancer*, **2024**, 5(8), 1250-1266.
[18]. Tamboli, A., Tayade, S., In-depth investigation of berberine and tropane through computational screening as possible dpp-iv inhibitors for the treatment of T2DM. *Journal of Pharmaceutical Sciences and Computational Chemistry*, **2025**, 1(1), 1-11.
[19]. Siddiqui, F., Makhloufi, R., Salah, M.E.S., Mohamed, E., Hojjati, M., Computational exploration of quinine and mefloquine as potential anti-malarial agents. *Journal of Pharmaceutical Sciences and Computational Chemistry*, **2025**, 1(2), 106-115.
[20]. Ahmed, S., Tabassum, P., Falak, S., Ahmad, A., Shaikh, M., Molecular docking and network pharmacology: Investigating vitis vinifera phytoconstituents as multi-target therapeutic agents against breast cancer. *Journal of Pharmaceutical Sciences and Computational Chemistry,* **2025**, 1(2), 116-134.
[21]. Jadhav, S., Dighe, P., Synthesis, in vitro evaluation, and molecular docking studies of novel pyrazoline derivatives as promising bioactive molecules. *Journal of Pharmaceutical Sciences and Computational Chemistry*, **2025**, 1(3), 190-209.
[22]. Zengin Kurt, B., Öztürk Civelek, D., Cakmak, E.B., Kolcuoğlu, Y., Şenol, H., Sağlık Özkan, B.m.N., Dag, A., Benkli, K., Synthesis of sorafenib– ruthenium complexes, investigation of biological activities and applications in drug delivery systems as an anticancer agent. *Journal of Medicinal Chemistry*, **2024**, 67(6), 4463-4482.
[23]. Patil, S.M., Asgaonkar, K., Bhandari, S., Edake, A.M., Patel, H., Patil, B., Ansari, I., Mahadik, I., Kamble, O., Gavande, N.S., Integrated computational approach for designing potent egfr-tk inhibitors: Qsar, docking, admet, and molecular dynamics studies. *Chemistry & Biodiversity*, **2025**, e202403150.
[24]. Saiyad, A.H., Godhani, D.R., Mehta, U.P., Parmar, K.P., Mehta, J.P., Patel, H.M., Ahmad, I., Synthesis, antimicrobial activity, molecular docking and md simulation studies, in silico adme investigations and

thermo-acoustic studies of heterocyclic dihydropyrimidine substituted 1, 3, 4-thiadiazole scaffolds. *Journal of Molecular Structure*, **2025**, 1321, 140083.
[25]. Muslim, R.F., Guma, M.A., Mahmood, A.A.R., Ahmad, I., Hirad, A.H., Patel, H., Synthesis, characterization, molecular modeling studies and bioactivity of a novel bicyclic compound of δ-lactam with oxazepine ring containing sulphur substitute using an economic method. *Journal of Computational Biophysics and Chemistry*, **2025**, 24(6), 733-750.
[26]. Saiyad, A.H., Godhani, D.R., Mehta, U.P., Parmar, K.P., Mehta, J.P., Patel, H.M., Ahmad, I., Synthesis, antimicrobial activity, molecular docking and md simulation studies, in silico adme investigations and thermo-acoustic studies of heterocyclic dihydropyrimidine substituted 1, 3, 4-thiadiazole scaffolds. *Journal of Molecular Structure*, **2025**, 1321, 140083.
[27]. Boulaamane, Y., Touati, I., Qamar, I., Ahmad, I., Patel, H., Chandra, A., Britel, M.R., Maurady, A., In silico discovery of dual ligands targeting mao-b and aa2ar from african natural products using pharmacophore modelling, molecular docking, and molecular dynamics simulations. *Chemistry Africa*, **2024**, 7(8), 4337-4359.
[28]. Ahmad, I., Patel, H., Mechanistic insights into the allosteric inhibition of egfr by dibenzodiazepinone ddc4002: A molecular dynamics, binding free energy, conformational motions and thermodynamic landscape approach. *In Silico Research in Biomedicine*, **2025**, 100048.
[29]. Amadei, A., Linssen, A.B., Berendsen, H.J., Essential dynamics of proteins. *Proteins: Structure, Function, and Bioinformatics*, **1993**, 17(4), 412-425.
[30]. Elsaman, T., Ahmad, I., Eltayib, E.M., Suliman Mohamed, M., Yusuf, O., Saeed, M., Patel, H., Mohamed, M.A., Flavonostilbenes natural hybrids from rhamnoneuron balansae as potential antitumors targeting aldh1a1: Molecular docking, admet, mm-gbsa calculations and molecular dynamics studies. *Journal of Biomolecular Structure and Dynamics*, **2024**, 42(6), 3249-3266.
[31]. Chaudhary, N., Aparoy, P., Application of per-residue energy decomposition to identify the set of amino acids critical for in silico prediction of cox-2 inhibitory activity. *Heliyon*, **2020**, 6(10).